

AMENDMENTS TO THE CLAIMS

Claim 1 (original) A method of synthesizing a target polynucleotide comprising:

- a) providing a target polynucleotide sequence;
- b) identifying at least one initiating polynucleotide present in the target polynucleotide of a), wherein the initiating polynucleotide comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide comprised of a 5' overhang and a 3' overhang;
- c) identifying a second polynucleotide present in the target polynucleotide of a), wherein the second polynucleotide is contiguous with the initiating polynucleotide and comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide comprised of a 5' overhang, a 3' overhang, or a 5' overhang and a 3' overhang, wherein at least one overhang of the second polynucleotide is complementary to at least one overhang of the initiating polynucleotide;
- d) identifying a third polynucleotide present in the target polynucleotide of a), wherein the third polynucleotide is contiguous with the initiating sequence and comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide comprised of a 5' overhang, a 3' overhang, or a 5' overhang and a 3' overhang, wherein at least one overhang of the third polynucleotide is complementary to at least one overhang of the initiating polynucleotide which is not complementary to an overhang of the second polynucleotide;
- e) contacting the initiating polynucleotide of b) with the second polynucleotide of c) and the third polynucleotide of d) under conditions and for such time suitable for annealing, the contacting

resulting in a contiguous double-stranded polynucleotide, wherein the initiating sequence is extended bi-directionally;

f) in the absence of primer extension, optionally contacting the mixture of e) with a ligase under conditions suitable for ligation; and

g) optionally repeating b) through f) to sequentially add double-stranded polynucleotides to the extended initiating polynucleotide through repeated cycles of annealing and ligation, whereby a target polynucleotide is synthesized.

Claim 2 (original) The method of claim 1, wherein the target polynucleotide sequence encodes a target polypeptide.

Claim 3 (original) The method of claim 2, wherein the target polypeptide is a protein

Claim 4 (original) The method of claim 3, wherein the protein is an enzyme.

Claim 5 (original) The method of claim 1, wherein the initiating polynucleotide sequence is identified by a computer program.

Claim 6 (withdrawn) The method of claim 5, wherein the computer program comprises the following algorithm.

Claim 7 (original) The method of claim 1, wherein the plus strand of the initiating, second or third polynucleotide is about 15 to 1000 nucleotides in length.

Claim 8 (original) The method of claim 1, wherein the plus strand of the initiating, second or third polynucleotide is about 20 to 500 nucleotides in length.

Claim 9 (original) The method of claim 1, wherein the plus strand of the initiating, second or third polynucleotide is about 25 to 100 nucleotides in length.

Claim 10 (original) The method of claim 1, wherein the minus strand of the initiating, second or third polynucleotide is about 15 to 1000 nucleotides in length.

Claim 11 (original) The method of claim 1, wherein the minus strand of the initiating, second or third polynucleotide is about 20 to 500 nucleotides in length.

Claim 12 (original) The method of claim 1, wherein the minus strand of the initiating, second or third polynucleotide is about 25 to 100 nucleotides in length.

Claim 13 (original) The method of claim 1, wherein the initiating polynucleotide is attached to a solid support.

Claim 14 (original) A method of synthesizing a target polynucleotide comprising:

- a) providing a target polynucleotide sequence derived from a model sequence;
- b) identifying at least one initiating polynucleotide sequence present in the target polynucleotide sequence of a), wherein the initiating polynucleotide comprises: 1) a first plus strand oligonucleotide; 2) a second plus strand oligonucleotide contiguous with the first plus strand oligonucleotide; and 3) a minus strand oligonucleotide comprising a first contiguous sequence that is at least partially complementary to the first plus strand oligonucleotide and second contiguous sequence which is at least partially complementary to the second plus strand oligonucleotide;
- c) annealing the first plus strand oligonucleotide and the second plus strand oligonucleotide to the minus strand

oligonucleotide of b) resulting in a partially double-stranded initiating polynucleotide comprised of a 5' overhang and a 3' overhang;

d) identifying a second polynucleotide sequence present in the target polynucleotide sequence of a), wherein the second polynucleotide sequence is contiguous with the initiating polynucleotide sequence and comprises: 1) a first plus strand oligonucleotide; 2) a second plus strand oligonucleotide contiguous with the first plus strand oligonucleotide; and 3) a minus strand oligonucleotide comprising a first contiguous sequence which is at least partially complementary to the first plus strand oligonucleotide and second contiguous sequence which is at least partially complementary to the second plus strand oligonucleotide;

e) annealing the first plus strand oligonucleotide and the second plus strand oligonucleotide to the minus strand oligonucleotide of d) resulting in a partially double-stranded second polynucleotide, wherein at least one overhang of the second polynucleotide is complementary to at least one overhang of the initiating polynucleotide;

f) identifying a third polynucleotide present in the target polynucleotide of a), wherein the third polynucleotide is contiguous with the initiating sequence and comprises: 1) a first plus strand oligonucleotide; 2) a second plus strand oligonucleotide contiguous with the first plus strand oligonucleotide; and 3) a minus strand oligonucleotide comprising a first contiguous sequence which is at least partially complementary to the first plus strand oligonucleotide and second contiguous sequence which is at least partially complementary to the second plus strand oligonucleotide;

g) annealing the first plus strand oligonucleotide and the second plus strand oligonucleotide to the minus strand oligonucleotide of f) resulting in a partially double-stranded second polynucleotide, wherein at least one overhang of the third polynucleotide is complementary to at least one overhang of the

initiating polynucleotide and not complementary to an overhang of the second polynucleotide;

h) contacting the initiating polynucleotide of c) with the second polynucleotide of e) and the third polynucleotide of g) under conditions and for such time suitable for annealing, the contacting resulting in a contiguous double-stranded polynucleotide, wherein the initiating sequence is extended bi-directionally;

i) in the absence of primer extension, optionally contacting the mixture of h) with a ligase under conditions suitable for ligation; and

j) optionally repeating b) through i) to sequentially add double-stranded polynucleotides to the extended initiating polynucleotide through repeated cycles of annealing and ligation, whereby a target polynucleotide is synthesized.

Claim 15 (withdrawn) A method for synthesizing a target polynucleotide, comprising:

a) providing a target polynucleotide sequence;

b) identifying at least one initiating polynucleotide present in the target polynucleotide of a), wherein the initiating polynucleotide comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide;

c) contacting the initiating polynucleotide under conditions suitable for primer annealing with a first oligonucleotide having partial complementarity to the 3' portion of the plus strand of the initiating polynucleotide, and a second oligonucleotide having partial complementarity to the 3' portion of the minus strand of the initiating polynucleotide;

d) catalyzing under conditions suitable for primer extension: 1) polynucleotide synthesis from the 3'-hydroxyl of the plus strand of the initiating polynucleotide; 2) polynucleotide synthesis from the 3'-hydroxyl of the annealed first oligonucleotide; 3) polynucleotide synthesis from the 3'-hydroxyl of the minus strand of the initiating polynucleotide; and 4) polynucleotide synthesis from the 3'-hydroxyl

of the annealed second oligonucleotide, wherein the initiating sequence is extended bi-directionally thereby forming a nascent extended initiating polynucleotide;

e) contacting the extended initiating polynucleotide of d) under conditions suitable for primer annealing with a third oligonucleotide having partial complementarity to the 3' portion of the plus strand of the extended initiating polynucleotide, and a fourth oligonucleotide having partial complementarity to the 3' portion of the minus strand of the extended initiating polynucleotide;

f) catalyzing under conditions suitable for primer extension: 1) polynucleotide synthesis from the 3'-hydroxyl of the plus strand of the extended initiating polynucleotide; 2) polynucleotide synthesis from the 3'-hydroxyl of the annealed third oligonucleotide; 3) polynucleotide synthesis from the 3'-hydroxyl of the minus strand of the extended initiating polynucleotide; and 4) polynucleotide synthesis from the 3'-hydroxyl of the annealed fourth oligonucleotide, wherein the extended initiating sequence is extended bi-directionally thereby forming a nascent extended initiating polynucleotide; and

g) optionally repeating e) through f) as desired, resulting in formation of the target polynucleotide sequence.

Claim 16 (withdrawn) The method of claim 15, wherein the target polynucleotide sequence encodes a target polypeptide.

Claim 17 (withdrawn) The method of claim 16, wherein the target polypeptide is a protein.

Claim 18 (withdrawn) The method of claim 17, wherein the protein is an enzyme.

Claim 19 (withdrawn) The method of claim 15, wherein the initiating polynucleotide is identified by an algorithm.

Claim 20 (withdrawn) A method of synthesizing a target polynucleotide comprising:

a) providing a target polynucleotide sequence;

b) identifying at least one initiating polynucleotide present in the target polynucleotide of a), wherein the initiating polynucleotide comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide comprised of at least a 5' overhang or a 3' overhang;

c) identifying a second polynucleotide present in the target polynucleotide of a), wherein the second polynucleotide is contiguous with the initiating polynucleotide and comprises at least one plus strand oligonucleotide annealed to at least one minus strand oligonucleotide resulting in a partially double-stranded polynucleotide comprised of a 5' overhang, a 3' overhang, or a 5' overhang and a 3' overhang, wherein at least one overhang of the second polynucleotide is complementary to the overhang of the initiating polynucleotide;

d) contacting the initiating polynucleotide of b) with the second polynucleotide of c) under conditions and for such time suitable for annealing, the contacting resulting in a contiguous double-stranded polynucleotide, wherein the initiating sequence is extended uni-directionally;

e) in the absence of primer extension, optionally contacting the mixture of e) with a ligase under conditions suitable for ligation; and

f) optionally repeating b) through e) to sequentially add double-stranded polynucleotides to the extended initiating polynucleotide through repeated cycles of annealing and ligation, whereby a target polynucleotide is synthesized.

Claim 21 (withdrawn) The method of claim 15, wherein the plus strand of the initiating, second or third polynucleotide is about 15 to 1000 nucleotides in length.

Claim 22 (withdrawn) The method of claim 15, wherein the plus strand of the initiating, second or third polynucleotide is about 20 to 500 nucleotides in length.

Claim 23 (withdrawn) The method of claim 15, wherein the plus strand of the initiating, second or third polynucleotide is about 25 to 100 nucleotides in length.

Claim 24 (withdrawn) The method of claim 15, wherein the minus strand of the initiating, second or third polynucleotide is about 15 to 1000 nucleotides in length.

Claim 25 (withdrawn) The method of claim 15, wherein the minus strand of the initiating, second or third polynucleotide is about 20 to 500 nucleotides in length.

Claim 26 (withdrawn) The method of claim 15, wherein the minus strand of the initiating, second or third polynucleotide is about 25 to 100 nucleotides in length.

Claim 27 (withdrawn) The method of claim 15, wherein the initiating polynucleotide is attached to a solid support.

Claims 28-37 (canceled)

Claim 38 (original) The method of claims 1, 14, 15 or 27, wherein the oligonucleotides are produced by synthesis on a automated DNA synthesizer.

Claims 39-52 (canceled)